

Targeting vasoactive peptides for managing calcific aortic valve disease

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Introduction

Calcific aortic valve disease (CAVD) represents a spectrum of disease spanning from mild aortic valve sclerosis to severe aortic stenosis (AS) with hemodynamic instability (1). CAVD is the most common valvular heart disease in Europe and North America and its prevalence is increasing rapidly due to the aging of the population. The prevalence of aortic stenosis is only about 0.2% among adults over 50 years of age, but increases to 9.8% over 80 years of age, with an overall prevalence of 2.8% in adults older than 75 years of age (2). Even without significant stenosis, aortic valve sclerosis is associated with a 50% increased risk of myocardial infarction and death from cardiovascular causes (1–3). Once symptoms, including angina, syncope, or heart failure are present in AS, valve replacement is the only effective treatment (1). The average survival without valve replacement is no more than 1.5–2 years after the onset of symptoms among patients with severe AS (2,4).

The pathogenesis of CAVD is initiated by endothelial injury, followed by lipid accumulation, infiltration of inflammatory cells, degradation or increased deposition of the extracellular matrix (ECM), neoangiogenesis, and extensive calcification combined with ossification (5–7). These processes are also contributing factors of vascular atherosclerosis but despite pathophysiological similarities, only about 50% of patients with CAVD have clinically significant atherosclerosis (8). To date there is no pharmacological treatment available to treat or even to slow down the disease progression of CAVD. Therefore, mechanisms of progression from an early inflammatory lesion to phenotype transformation of valve myofibroblasts and then to the end stage of severe valve calcification have been intensively characterized to reveal novel targets for managing the disease.

In the present review, we discuss the role of vasoactive peptides in CAVD. We summarize the current knowledge of renin-angiotensin system (RAS) counterparts, chymase, cathepsin G, bradykinin, and its receptors as well as endothelin (ET), natriuretic peptide and apelin-APJ system in the pathogenesis of aortic valve calcification. In addition, we discuss potential strategies to target the vasoactive peptides for the development of pharmacological treatment and diagnosis to CAVD.

The renin-angiotensin system (RAS)

During the last decades the focus of research has been changed from systemic and endocrine RAS to autocrine and paracrine effects of tissue RAS (9). Tissue RAS functions by inducing fibroblast proliferation, expression of proinflammatory mediator ET-1

(10) and ECM deposition, suggesting that paracrine/ autocrine RAS may play a major role in the pathogenesis of aortic valve stenosis. In addition to the classical RAS (renin, Ang I, angiotensin converting enzyme [ACE], Ang II, and angiotensin II receptors type 1 and 2 [AT₁ and AT₂], newer components such as (pro)renin receptor, ACE2, its product angiotensin (1-7) [Ang-(1-7)] and its receptor Mas proto-oncogene have been recently discovered providing potential targets for pharmacological therapies. So far, RAS has been the major target for drugs in attempt to seek therapeutic strategies for patients with CAVD and several clinical trials have evaluated the effect of angiotensin receptor blockers (ARBs) and angiotensin convertase enzyme inhibitors (ACEIs) on the progression of CAVD.

Angiotensin II, AT₁ and AT₂ receptors

The functional effects of Ang II are mediated via two receptor isoforms AT₁ and AT₂. Although AT₁ shares structural homology with AT₂, they are functionally distinct and differentially distributed (11,12). The classical systemic effects of Ang II (vasoconstriction, cardiac hypertrophy, ECM formation, and increased release of aldosterone) are mediated via AT₁ (13). According to theory, the beneficial effects on cardiovascular system (antifibrotic effect, antihypertrophic effect, vasodilating effect and opposing the antinatriuretic effect) are mediated via AT₂ receptor (13). Several studies have described the existence of local RAS in aortic valves (14–17). AT₁ receptor mRNA levels are described to be both increased (15) and unchanged (16) in stenotic aortic valves. These results may be explained by the interindividual variability in the abundance of the AT₁ receptor in the valvular tissue since in one study AT₁ receptor was detected in only less than 20% of nonstenotic valves but in 75% of stenotic valves (14). Even though a decrease in gene expression levels of AT₂ receptor was seen in AS valves when compared with normal aortic valves, the AT₂-receptors were not detected by immunohistochemistry in normal or calcified valves (16). Ang II have been shown to promote aortic valve thickening independent of elevated blood pressure in apolipoprotein-E deficient mice (18), and induce vascular calcification *in vitro* and *in vivo* through receptor activator of nuclear factor- κ B ligand (RANKL) system activation (19). In addition, RANKL activated RAS, especially ACE and AT1 receptor, providing RANKL as a novel therapeutic option to inhibit RAS and prevent vascular calcification. Previously, RANKL, a component of emerging regulatory pathway (nuclear factor- κ B (RANK), RANKL, and osteoprotegerin (OPG)) for vascular calcification (20), has been reported to contribute to vascular calcification by decreasing the calcification inhibitor, matrix Gla protein, in vascular smooth muscle cells, and elevating bone morphogenetic protein-2 (BMP-2) expression in endothelial cells (19).

Three retrospective studies have been published according to which ARBs are useful in the treatment of AS (Table 1). Japanese Aortic Stenosis Study (JASS) showed that initiation of ARB treatment during the early stage of the CAVD may be effective in slowing the progression of AS (21),

and in a study with hypertensive patients, ARB (but not ACEI) treatment was associated with slower progression of CAVD (22). In another retrospective study, the use of ARBs was associated with a lower remodelling score of stenotic aortic valves (23). However, the treatment with candesartan for five months in patients with clinically significant AS did not have beneficial effects on mortality or left ventricular mass and function (24) (Table 2). Additionally, treatment of cholesterol-fed rabbits with olmesartan has been associated with decreased macrophage infiltration and reductions in osteopontin and ACE in aortic valves (25). Unfortunately, no hemodynamic valvular measurements of AS progression were performed in that study (25). In addition, hemodynamic factors and effects of ARB fimasartan on LV remodeling is being investigated in ongoing study in patients with asymptomatic moderate to severe AS as well as effect of telmisartan and losartan in bicuspid aortic valve patients (Table 2).

Angiotensin converting enzyme (ACE), ACE2 and Mas receptor

ACE is a metalloproteinase that hydrolyzes angiotensin I into Ang II. Additionally, there are two other

Table 1. Retrospective cohort studies of angiotensin converting enzyme inhibitor (ACEI) and angiotensin receptor blocker (ARB) treatments in calcific aortic valve disease (CAVD).

Reference	Main method of patient classification	Study groups	Number of subjects	Age	Mean follow-up time	Conclusions	Notes
(6)	Echocardiography	No ACEI ACEI	109 102	69 ± 11 70 ± 10	24 ± 18 mo.	ACEI not associated with CAVD progression	No effect found in all ACEI doses and types, or combination with statin
(14)	Serial EBT	No ACEI	80	67 ± 98	2.5 years	ACEI use associated with lower rate of CAVD progression	
(21)	Echocardiography	Early-stage AS Late-stage AS	43 157 399	69.3 ± 8.4 71.1 ± 8.0 74.7 ± 9.2	2.8 years	Non-use of ARBs correlated with faster progression of early-stage CAVD	
(23)	Valve histology		208	69 ± 9	Duration of ACEI/ARB treatment unavailable	ARB treatment associated with lower valve remodelling score	Explanted end-stage valves used
(22)	Echocardiography	No hypertension, no RAS treatment Hypertension, no RAS treatment Hypertension, ACEI treatment Hypertension, ARB treatment	N/A 92 77 113 56	61 ± 18 73 ± 12 71 ± 11 72 ± 11	6.2 ± 2.4 years	Use of ARBs but not ACEI associated with slower progression of CAVD	Use of ARBs but not ACEI also associated with reduced mortality

AS: aortic stenosis; EBT: electron beam computed tomography; mo: months; RAS: renin-angiotensin system; N/A: not applicable.

enzymes, chymase and cathepsin G, capable of increasing Ang II amounts locally in stenotic aortic valves (15,30). ACE2 is thought to counteract actions of Ang II by hydrolysing Ang II to Ang-(1-7), which in turn has opposite effects to those of Ang II by mediating beneficial antifibrotic, antihypertrophic, antiproliferative and anti-inflammatory effects (9,13,31).

ACE is present in aortic valvular lesions, where it colocalizes with its product, Ang II, and with retained plasma lipoproteins (14). In calcified valves, both ACE mRNA levels and protein activity of ACE are increased (15), whereas components of ACE2-Ang-(1-7)-Mas-axis were reported to be downregulated in stenotic aortic valves (17). Due to decreased ACE2 mRNA levels in stenotic valves, the disequilibrium between ACE and ACE2 expressions may play a key role in fibrocalcific process in which Ang II generation by ACE, chymase and cathepsin G is increased (14,15,17,30). Interestingly, these enzymes can also inactivate the antifibrotic bradykinin (50). Furthermore, Ang-(1-7) has antiangiogenic properties (32) suggesting that decreased production of Ang-(1-7) may, in part, contribute to the angiogenesis in fibrocalcific process. All in all, ACE inhibition may result in antiatherosclerotic effects, reduced neointimal formation and improved endothelial function and plaque stabilization (33), and ACEIs are on theoretical level likely to have beneficial effects among CAVD patients (34).

There are several studies evaluating the effect of ACEIs in patients of AS (Tables 1–2). Indeed, beneficial effects have been reported in a retrospective report concerning ACEI treatment and lowered aortic valve calcium (AVC) accumulation, as measured by electron beam computed tomography (35). In three clinical trials, the beneficial effects of ACEIs can be explained by improved hemodynamics (26–28) (Table 2). In addition, in a recent observational study of more than 2000 patients with AS, the use of RAS inhibitors was associated with a significantly lower risk of death and other serious cardiovascular events in AS, even in patients with severe valve obstruction (36). In one prospective, double-blind, controlled trial the ramipril group showed a slower rate of progression of the AS (valve area 0.0 cm² in the ramipril group vs. –0.2 cm² in the placebo arm) but without statistical significance (29). Furthermore, there is an ongoing study of effects of captopril or trandolapril on hemodynamic and functional parameters in patients with severe AS. As regards human studies, no prospective large trials of ACE have been published to date. High blood pressure can be considered as a major determinant of valvular disease progression, maybe through mechanical stretching of

Study	Intervention	No of patients	Duration	CAVD status	Main outcome variables	Results	Reference/ Clinicaltrials.gov identifier
SCOPE-AS ^a	Enalapril vs. placebo	56	1 month (3 month follow-up for selected patients)	Symptomatic severe	Development of hypertension, Borg dyspnea index, 6-min walk distance, off-target drug effects, NYHA-class changes, echocardiographic parameters	Improvement in NYHA class, Borg index and 6-min walk distance. Benefit greatest in patients with regurgitant valve lesions	(26)
Effects of angiotensin converting enzyme inhibitors in hypertensive patients with aortic valve stenosis: a drug withdrawal study ^a	Various ACEIs, cross-over design	20	Not reported (withdrawal study)	Asymptomatic AS	Hemodynamic and functional variables	Reduction in afterload, compensated by increase in trans-valvular pressure gradient. Hemodynamic variables improved in most hypertensive patients.	(27)
Short-term hemodynamic effect of angiotensin-converting enzyme inhibition in patients with severe aortic stenosis ^a	Trandolapril vs. placebo	44	8 weeks	Severe AS	Acute hemodynamic effects of ACEI, change in systemic BP, SAC, Zva, NT-proBNP, exercise capacity	Reduction in left ventricular end systolic volume and NT-proBNP	(28)
RIAS ^a	Ramipril vs. placebo	100	1 year	Moderate or severe asymptomatic AS	LVM, systolic velocity, progression of CAVD by cardiac magnetic resonance	Reduction in LVM, preserved systolic pressure, non-significant reduction in progression of CAVD	(29)
ROCK-AS ^a	Candesartan vs. placebo	51	5 months	Severe AS	Mortality, LV mass and function, 6-min walking test, Nt-proBNP		(24)
ACCESS ^b	Captopril or trandolapril vs placebo	64	8 weeks	Severe AS	Hemodynamic and functional parameters		NCT00252317
ALFA ^b	Fimasartan vs. placebo	100	1 year	Moderate to severe AS	Change of VmaxO ₂ in cardiopulmonary exercise test, various hemodynamic parameters, LVM, hospitalization, mortality		NCT01589380
BAV study ^c	Telmisartan vs. placebo. Parallel arm tests atenolol vs. placebo.	85	5 years	BAV, no CAVD at baseline	Change from baseline in ascending aorta size, as evaluated by MRI; rate of change in ascending aorta size evaluated by (TEE)		NCT01202721
The effect of losartan in bicuspid aortic valve patients. ^b	Losartan (open label, single group)	25	1 year	BAV, no CAVD at baseline	Inflammatory blood markers		NCT01390181

Status of the study ^acompleted, ^bunknown, and ^congoing.

AS: aortic stenosis; NYHA: New York Heart Association; BP: blood pressure; SAC: systemic aortic compliance; NT-proBNP: N-terminal pro-brain natriuretic peptide; Zva: valvuloarterial impedance; LVM: left ventricular mass; BAV: bicuspid aortic valve; MRI: magnetic resonance imaging; TEE: transthoracic echocardiography.

ACCESS: Acute Haemodynamic Effects of Treatment With Angiotensin Converting Enzyme (ACE)-Inhibitors in Patients With Symptomatic Aortic Stenosis; ALFA: A Randomized Trial of Angiotensin Receptor blocker, Fimasartan, in Aortic Stenosis; BAV: Beta Blockers and Angiotensin Receptor Blockers in Bicuspid Aortic Valve Disease Aortopathy; RIAS: A prospective, double-blind: randomized controlled trial of the angiotensin-converting enzyme inhibitor Ramipril In Aortic Stenosis; ROCK-AS: The Potential of Candesartan to Retard the Progression of Aortic Stenosis; SCOPEAS: Safety and efficacy of angiotensin-converting enzyme inhibitors in symptomatic severe aortic stenosis: Symptomatic Cardiac Obstruction-Pilot Study of Enalapril in Aortic Stenosis.

In addition, increased vascular afterload (ventricular wall stress) induces left ventricular hypertrophy and dysfunction, and this together with chronic valvular and vascular pressure overload is a major cause of the morbidity and mortality of CAVD (37). Thus, some beneficial effects of ACE inhibitors, and ARBs as well, on clinical outcomes in patients with AS, could be attributed to lowering of blood pressure.

There might also be differences between ACE inhibition and AT₁ antagonism as pharmacologic approach for calcified aortic valve disease. In several studies ACE inhibitors have modulated various components of the atherosclerotic process by inhibiting Ang II formation and by reducing breakdown of anti-fibrotic bradykinin (33). Ang II increases lipid peroxidation and oxyradical formation, stimulates the expression of proinflammatory genes, such as chemoattractant protein and leucocyte adhesion molecules, resulting in endothelial dysfunction (14,15,17,30). In addition, Ang II induces vascular smooth-muscle proliferation (38). By favoring the balance between Ang II and bradykinin, ACEIs are likely to improve endothelial function and counteract initiation and progression of atherosclerosis (33). However, quantitative differences do exist among ACEIs. Ramipril and perindopril are highly lipophilic and have strong enzyme-binding capabilities; such ACE inhibitors may probably provide greater penetration into the atherosclerotic plaque in valvular location as well. Interestingly, hemodynamic retardation of AS, concomitantly with reduction in calcification, macrophage infiltration, redox stress and improvement in endothelial function has been reported with ramipril treatment in a model of AS in New Zealand white rabbits (39).

Both Mas and AT₂-receptor mRNA levels are both significantly downregulated in stenotic aortic valves (17). Since, Ang II-forming enzymes are upregulated and AT₁-receptor protein levels are increased in stenotic valves (14,15), the downregulation of both Mas and AT₂-receptor expression (16,17), via unopposed AT₁-receptor activation, may augment the profibrotic and pro-inflammatory effects of RAS. Furthermore, Mas and AT₂-receptor activation induce eNOS activation (40), and mutant mice lacking the Mas exhibit impaired endothelial function, decreased NO production and lower endothelial NO synthase (eNOS) expression (40). In humans, expression of Mas receptor is mainly restricted to endothelial cells (41), so Mas, along with the AT₂, may contribute to the downregulation of eNOS and endothelial dysfunction in calcific aortic valve disease as well. Moreover, the first selective AT₂ agonist compound 21 (C21) has been shown to have anti-inflammatory effects (13,42) so it would be interesting to see whether AT₂ agonism could prevent inflammatory response related to disease progression of AS.

Renin, prorenin and (pro)renin receptor

Renin is a proteolytic enzyme synthesized and secreted as an inactive proenzyme, prorenin. The only known substrate for renin is angiotensinogen, which is cleaved by renin to form angiotensin I (43). A specific human renin receptor, (P)RR, binds both prorenin and renin and shows a dual function. First, binding of prorenin to (P)RR leads to its activation and to generation of Ang II locally (43). Secondly, binding of renin or prorenin to (P)RR results in the activation signaling pathways associated to profibrotic and proliferative actions as well as endothelial cell function and neovascularization, independent of Ang II production (43–45).

Both renin and prorenin gene expression have been reported to be downregulated in stenotic aortic valves compared to normal valves, whereas no change in (P)RR mRNA levels has been noted (17). Instead, a reduced amount of (P)RR positivity was seen in the valvular endothelial cells of stenotic valves but more in neovessels, consistent with proangiogenic effect of (P)RR (17). Prorenin receptor ((P)RR) is the first counterpart receptor of tissue

RAS, therefore (P)RR antagonism as a strategy would lead lowered activity of whole RAS cascade downwards. One important aspect of (P)RR is that it appears to be a vital component of the wnt/b- catenin pathway (46). With its component, low-density lipoprotein receptor-related protein 5 (Lrp5), this path- way seems to be upregulated in the valvular tissue of rabbit CAVD (47), and is a known regulator of osteo- genic differentiation (48). These raise the intriguing possibility that inhibition of (P)RR may also disturb the wnt/Lrp5/b-catenin-mediated osteogenic differenti- ation of valvular interstitial cells.

Bradykinin and bradykinin receptors

Kinin peptides act via two types of kinin receptors: the bradykinin B1 receptor (B1R) and the bradykinin B2 receptor (B2R). The cardioprotective effect of stimula- tion of B2R trigger the production of nitric oxide (NO) and exerts anti- proliferative and anti-hypertrophic effects on myocytes and fibroblasts (49). In stenotic valves, increased expression of B1R and B2R has been reported (50). Furthermore, exposure to tumor necrosis factor- α (TNF α) increased gene expression of B1R and B2Rin valvular cell culture environment (50). Ang II generation by ACE, chymase and cathepsin G is increased in AS valves (14,15,17,30). Interestingly, these enzymes can also inactivate the antifibrotic bradykinin (31,51). Conversely, bradykinin counteracts the nega- tive action of Ang II and improves endothelial function by increasing expression and activity of the constitu- tive NO synthase (50). Bradykinin also inhibits the expression of monocytes and adhesion molecules (50), and has an antiproliferative effect (49,52). In addition, in inflammatory conditions, bradykinin receptors B1 and B2 can couple to activate NF- κ B signaling through Akt and/or ERK1/2 pathways, which leads to inhibition of osteoblastic differentiation with subsequent activa- tion of osteoclast formation (53). Consequently, brady- kinin receptor specific agonists and/or antagonists could be therapeutic agents also in development of CAVD. Additionally, neutral endopeptidase (NEP or neprilysin), another enzyme capable to degrading bradykinin, is upregulated in stenotic valves (50). These findings suggest that downregulation of brady- kinin contributes to the shift of balance between fibrotic and antifibrotic factors promoting fibrosis in calcific aortic valve stenosis (17). Consequently, inhibit- ing ACE, chymase, cathepsin G, and NEP that all con- tribute in producing Ang II by inhibiting bradykinin might lead to success as pharmacological CAVD therapy.

Endothelin and endothelin receptors

Four structurally different endothelin (ET) isoforms have been described (i.e., ET-1, ET-2, ET-3, ET-4). Mature ET-1 is formed from pre-pro-ET-1 via an inter- mediate big ET-1 by a family of endothelin converting enzymes (ECEs) and other enzymes such as chymases, and endopeptidases (54). The biological effects of ET-1 are mediated by ET_A and ET_B receptors, both of which are expressed in aortic valves. ET_A receptor expression is increased in stenotic valves whereas no changes in ET_B expression was reported (55). In addition, increased plasma big ET-1 levels have been reported in patients with AS (56). In stenotic aortic valves, ET-1 protein is increased without increase in ET-1 mRNA levels, likely reflecting ET_B receptor mediated up-take of ET-1 from circulation (55). However, the levels of ET_B receptors were similar between control and sten- otic valves in the recent study (55). Another possibility would be the changes in the degradation of ET-1. This seems, however, unlikely due to the increase in NEP activity in stenotic aortic valves (15). The pathophysio- logical significance of increased ET-1 peptide levels in aortic valve stenosis may be further

enhanced by the concomitant upregulation of ET_A receptor gene (55). Since, continued stimulation of cells with agonists generally result in a down-regulation of receptors, one would expect either ET_A or ET_B or both receptors to be down-regulated due to higher ET-1 peptide levels in stenotic valves. So it is possible that the net effect of ET-1 can be considered to be determined by receptor localization and the balance between ET_A and ET_B receptors (57). As increased ET-1 and ET_A protein levels have been documented in stenotic aortic valves (55), particularly ET_A receptor antagonism could potentially reduce the actions of ET-1 and even slow down the development of CAVD.

Interestingly, ET-1 was found to regulate calcification in both *in vivo* and in cultured vascular smooth muscle cells (58). Moreover, administration of bosentan attenuated vascular calcification induced by vitamin D3 plus administration of nicotine in rats, but whether ET-1 has a role in development in CAVD is not known (58). Experimental studies have established a role for vitamin D metabolites in pathways that are integral to cardiovascular function and disease, including inflammation, thrombosis, and the renin-angiotensin system and low levels of vitamin D are associated with cardiovascular diseases (59). Interestingly, VDR deficiency and diets low in vitamin D promote aortic valve and aortic vessel calcification in the VDR^{-/-} and LDLR^{-/-} mouse models (60). The vascular calcification in this study was associated with an up-regulation of osteoblast transcription factors which could have triggered the differentiation of vascular cells into osteoblast-like cells (60).

The effect of dualistic ET_A/ET_B inhibition by tezosentan on ET-1 uptake was recently studied in cultured human aortic valve tissue explants (61). Promisingly, tezosentan reduced the uptake of ET-1 to valve tissue more effectively in sclerotic aortic valve cusp explants than in macroscopically normal sections (61). However, it remains to be determined whether ET receptor antagonists might have beneficial effects in slowing down the progression of AS. Experimentally, ET_A receptor blockade prevents endothelial dysfunction and structural vascular changes in atherosclerosis (54). On the other hand, the vasodilating and blood pressure lowering properties of ET receptor antagonists might present a clinical problem in AS. In patients with heart failure, ET_A receptor antagonists (e.g., tezosentan) have controversial effects, although neurohormones and natriuretic peptides decreased favorably by treatment (62). In addition to ET-1, NO signaling pathway is involved in aortic valve calcification since both eNOS and iNOS are expressed in human aortic valves, and eNOS gene expression is downregulated in stenotic valves (55). Thus, imbalance between ET_A and ET_B receptors is potentiated by downregulated eNOS gene expression, similarly to endothelial dysfunction in atherosclerosis (57).

Apelin – APJ system

Apelin, an adipokine, and its receptor APJ are expressed in several tissues including the heart, vasculature and aortic valves (16,63). The proposed cardiovascular effects of the apelin-APJ system are opposite to the effects of the RAS. ACE2, breaking down Ang II to Ang (1-7), acts on apelin as well, suggesting a dynamic interaction between apelin and Ang II pathways (64). Interestingly, non-activated APJ suppresses AT₁, whereas apelin-activated APJ acts conversely (65).

In stenotic aortic valves, there are increased mRNA and protein levels of apelin which are potentiated by the up-regulation on APJ gene (16). Therefore, the upregulated apelin-APJ axis may also act as a compensatory mechanism ameliorating the harmful effects of AT₁ receptor activation in the pathogenesis of aortic valve stenosis. In endothelial cells, apelin is expressed primarily in cells at sites of active vascular growth (66), and APJ receptor may play an

important role in angiogenesis (67). *In vitro*, apelin promotes chemotaxis in human endothelial cells (68), a well-established phenomenon in the plaque formation in stenotic aortic valves (69). In addition, apelin has been reported to stimulate proliferation and to suppress apoptosis of mouse osteoblastic cell line (70), and importantly, apelin was shown to attenuate the osteoblastic differentiation of aortic valve interstitium cells (VICs) via the extracellular signal-regulated kinase (ERK) and phosphatidylinositol-3 kinase (PI3-K)/Akt pathway (71). Consequently, APJ receptor antagonists might be beneficial in the treatment of aortic valve stenosis by suppressing chemotaxis, angiogenesis and osteoblast activity. Apelin can also block a number of Ang II-related pathological processes associated with atherosclerosis (72) and inhibit fibrosis (73). It could be negatively regulated by miR125b (74), which are upregulated in stenotic valves (75). So far, there are no drugs targeting the apelin/APJ system available. By development of suitable agonists/antagonists, it will be intriguing to evaluate the impact of drug treatment targeting apelin/APJ system on CAVD pathology.

Natriuretic peptides

Natriuretic peptide family consists of three members, namely ANP (atrial natriuretic peptide), BNP (B-type natriuretic peptide) and CNP (C-type natriuretic peptide), whose biological effects are mediated by specific cell surface guanylate cyclase (GC)-linked receptors. ANP, BNP and CNP are produced as pro-forms and they are converted into mature peptides by proteolytic processing of the respective precursor molecules. Corin has been identified as a proANP- and proBNP- converting enzyme (76), whereas furin processes proCNP to its mature form (77).

All natriuretic peptides, their receptors as well as their processing enzymes are expressed in aortic valves (78). The CNP system, i.e., CNP, furin and target receptor GC-B, has been reported to be downregulated in stenotic valves, when compared with non-calcified aortic valves while no changes in ANP and BNP were seen (78). Degradation of CNP does not exist only via target receptor GC-B or clearance receptor natriuretic peptide receptor-C (NPR-C), but via proteolytic processing by NEP, which has been reported to be upregulated in AS both in mRNA and protein level (50).

Interestingly, CNP has a role in the bone growth (79,80), and it seems to inhibit vascular calcification (81), fibrosis (82) and inflammation (83), which suggests its downregulation may be a contributing factor in several underlying processes of CAVD. In one study, CNP attenuated calcification partly via a cGMP/protein kinase G pathway both in rat calcified aortas and cultured vascular smooth muscle cells affecting protein levels of osteopontin and bone morphogenetic protein (81). In addition, CNP has been shown to suppress calcified aggregate formation in (VICs) *in vitro*, as well as inhibit differentiation of VICs to osteoclasts and myofibroblasts when cultured under osteogenic and myofibroblastic conditions, respectively (84). Furthermore, CNP expression was stimulated by simvastatin in VICs grown in myofibroblastic conditions, whereas small interfering RNA knockdown of NPR-C significantly reduced the antifibrotic effect of simvastatin, suggesting that statins may act in part via CNP/NPR-B autocrine/paracrine signaling in the aortic valve (84).

The studies of mice with endothelial-specific deletion of CNP (ecCNP KO mouse) indicated that endothelium derived CNP could contribute to reversing the development of atherosclerotic lesions (85) and intervention with endothelium derived CNP could ameliorate fibrosis by reducing the activity and release of several matrix metalloproteinases (MMPs), including MMP-9, (86), whose mRNA expression and activation is increased in calcific valves (87). However, if CNP agonism with valvular targeted delivery would be considered as pharmacological intervention, valvular target receptors should be

CNP-degrading enzymes, as is the case with LCZ696 (sacubitril/valsartan). Combined neprilysin inhibition with valsartan (sacubitril p valsartan) seems to be interesting dualistic strategy with solid theory of benefits of ARB as well as prolonging effect of natriuretic peptides. Sacubitril is a first-in-class angiotensin receptor neprilysin inhibitor, converted by esterases to LBQ657, which inhibits neprilysin, the enzyme responsible for the degradation of the natriuretic peptides and many other vasoactive peptides (88). The neprilysin inhibition in combination with ARB therapy could theoretically improve prognosis in patients with CAVD due to its beneficial effects on congestive heart failure (89).

Finally, BNP is the most investigated circulating biomarker for AS, and it has been shown to be related to severity and functional status of the disease (90,91). In a recent study, BNP clinical activation was detected as a predictor of long-term mortality in patients with moderate or severe AS (92), whereas in a large prospective cohort NT-proBNP had a poor diagnostic value for severe symptomatic AS in elderly patients (91). Consequently, BNP has not yet been shown to provide sufficient incremental value in risk stratification in managing patients with AS (1,93).

Conclusions

Several components of vasoactive peptide systems are expressed in aortic valves with significant up/downregulation in CAVD (Table 3). So far, local RAS is the most studied vasoactive system, and the expression of its components may contribute to ACE/AngII/AT1-receptor-mediated fibrosis, proliferation and inflammation in AS (Figure 1). Clinical trials have shown beneficial effects in AS patients managed with ARB but not with ACEI (Tables 1–2). Although vasoactive factors have been intensively studied at the molecular level, there is lack of pharmacological agents to manipulate these systems. Nevertheless, renin, (P)RR, and endothelin (ETA/ETB) receptor antagonists should be studied in more detail in prospective clinical studies in CAVD patients.

Disclosure statement

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